Simvastatin prevents inflammation-induced aortic stiffening and endothelial dysfunction

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Both acute and chronic inflammation are associated with aortic stiffening and endothelial dysfunction.
- Statins have been shown to reduce inflammation and arterial stiffening and to improve endothelial function in patients with chronic inflammation.
- In a model of acute-inflammation, statins have been shown to prevent endothelial dysfunction, but the effect on aortic stiffening is unknown.

WHAT THIS STUDY ADDS

- This study demonstrates, for the first time, that pre-treatment with simvastatin prevents inflammation-induced aortic stiffening, as well as endothelial dysfunction, in a cohort of healthy individuals.
- It also shows that simvastatin prevents the inflammation-induced reduction in concentrations of apolipoprotein A-I.

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AIMS

The aim of this study was to determine whether simvastatin would protect against inflammation-induced aortic stiffening and endothelial dysfunction.

METHODS

Aortic pulse wave velocity (aPWV) and flow-mediated dilatation (FMD) were assessed three times, at baseline, after a 14 day administration of simvastatin or placebo and 8 h after *Salmonella typhi* vaccination in 50 healthy subjects.

RESULTS

Following vaccination there was a significant increase in aPWV in the placebo group (5.80 ± 0.87 vs. 6.21 ± 0.97 m s⁻¹, 95% CI 0.19, 0.62, $P = 0.002$) but not the simvastatin group (5.68 ± 0.73 vs. 5.72 ± 0.74 m s⁻¹, 95% CI $-0.19, 0.27$, $P = 0.9$; $P = 0.016$ for comparison). Whereas FMD response was reduced in the placebo group (6.77 ± 4.10 vs. $5.27 \pm 2.88\%$, 95% CI $-2.49, -0.52$, $P = 0.02$) but not in the simvastatin group (7.07 ± 4.37 vs. $7.17 \pm 9.94\%$, 95% CI $-1.1, 1.3$, $P = 0.9$, $P < 0.001$ for comparison). There was no difference in the systemic inflammatory response between groups following vaccination. However, there was a significant reduction in serum apolipoprotein A-I (Apo A-I) in the placebo, but not in the simvastatin, group.

CONCLUSIONS

Simvastatin prevents vaccination-induced aortic stiffening and endothelial dysfunction. This protective mechanism may be due to preservation of the Apo A-I lipid fraction, rather than pleiotropic anti-inflammatory effects of statins.

Introduction

Inflammation is thought to play an important role in the pathogenesis of atherosclerosis [1]. Serum concentrations of the acute phase reactant C-reactive protein (CRP) are associated with future cardiovascular risk in both healthy subjects and those with established coronary heart disease [2, 3]. Interleukin-6 (IL-6), which induces CRP production, can directly impair endothelial function, and plasma IL-6 concentrations are also positively associated with cardiovascular risk [4] [5]. Moreover, subjects with chronic, high-grade, systemic inflammatory conditions, such as rheumatoid arthritis, are at substantially increased cardiovascular risk [6]. Acute, systemic inflammation may also lead to a short-term increase in the risk of cardiovascular events. Indeed, the incidence of acute coronary events is increased following inflammatory insults, such as acute respiratory tract infections [7], and up to 10% of all strokes may be associated with preceding bacterial infections [8]. Surgery is also associated with an increased risk of myocardial infarction for a number of weeks following the initial intervention [9].

Although the exact mechanisms by which inflammation leads to increased cardiovascular risk are unclear, experimental data indicate that endothelial dysfunction [10] and arterial stiffening [11] may be important. Both are established surrogate markers of cardiovascular risk [12], and are thought to play a role in the development of cardiovascular disease [13, 14]. Perhaps a better way to gain a clearer understanding as to the effects of inflammation on arterial stiffness and endothelial dysfunction would be to induce a modulating factor. One approach would be to take a 'healthy population' and induce an inflammatory response. This can be achieved by using the 'model' of acute inflammation induced by typhoid vaccine.

Using this experimental, clinical model of acute inflammation in healthy volunteers, several groups have demonstrated that vaccination leads to endothelial dysfunction of conduit and resistance vessels within 8 h [10, 15, 16]. Recently Vlachopoulos *et al.* extended these observations and demonstrated that vaccination also leads to transient aortic stiffening which can be prevented by pre-treatment with aspirin [11]. Other studies have shown that 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors (statins), protect endothelial dysfunction induced by endotoxin [16] and typhoid vaccination [17] but whether statins would also protect against arterial stiffening in unclear.

We hypothesized that acute, systemic inflammation would lead to both endothelial dysfunction and concomitant arterial stiffening in healthy individuals, and that the HMG CoA reductase inhibitor, simvastatin, would protect against these effects. Acute changes in inflammatory and lipid markers following vaccination were also examined to investigate the potential underlying mechanisms.

Methods

Study population

This was a randomized, double-blind, placebo-controlled study. Fifty healthy volunteers aged 20–40 years were recruited and studied at the Vascular Research Clinic, Addenbrooke's Hospital, Cambridge, UK. All subjects reported to be free from cardiovascular risk factors, including hypertension, and were not taking any medication. Subjects had not received a typhoid vaccine within 6 months or other vaccine within 3 months. The Local Research Ethics Committee approved the study and all participants gave written informed consent. The procedures followed were in accordance with institutional guidelines and the Declaration of Helsinki. All studies were performed in a quiet, temperature-controlled room. Studies were performed in the morning, except on the day 15, when measurements were done in the afternoon, 8 h post-vaccination. Subjects were asked to fast and withdraw from smoking and caffeine use from midnight before each visit. Venous blood was drawn for measurement of glucose, lipid and cytokine profiles. Baseline endothelial function and arterial stiffness were assessed, and subjects were then randomized to take 40 mg simvastatin at night or placebo (ratio 1:1). Randomization was performed using a randomization plan generator (<http://www.randomization.com>). Subjects returned to the department for repeat measurements on day 14 and day 15. In the morning of day 15 subjects received an intramuscular injection of typhoid vaccine and were studied 8 h later (Figure 1). On day 15, subjects were given a breakfast following the vaccine, allowing a 7 h fast before the measurements. Caffeine or tobacco was not allowed during day 15.

Measurement of arterial stiffness

Augmentation pressure, augmentation index (AIx), central blood pressure, and brachial (bPWV) and aortic pulse wave velocity (aPWV) were determined by waveform analysis using the SphygmoCor system (Atcor Medical, Sydney, Australia) as described in detail previously [18].

Measurement of conduit vessel endothelial function

Endothelial function was determined using high-resolution vascular ultrasound (Acuson XP 128/10) to record the diameter changes in the brachial artery to increased blood flow generated during reactive hyperaemia (FMD) and glyceryl trinitrate (GTN) as described previously [19] and is a highly reproducible technique in our laboratory.

Inflammatory stimuli

Salmonella typhi capsular polysaccharide vaccine 0.025 mg (Typhim Vi, Pasteur Merieux MSD) was injected into the gluteus muscle on the morning of day 15. Using a digital

Figure 1

Schema of the study protocol. This was a randomized, double-blind, placebo-controlled study. Following the baseline measurements on day 1, patients were randomized to receive either simvastatin 40 mg or placebo. On day 14 the effect of the drug was assessed on the haemodynamics and biochemical markers. In the morning of day 15 subjects were given typhoid vaccination, following that hourly blood tests were taken and at 8 h post-vaccination haemodynamics were assessed again. Visit to unit (■); 14 days treatment simvastatin/placebo – Treatment phase (□)

tympanic thermometer (Braun, ThermoScan), temperature was measured before and each hour for 8 h following the typhoid vaccine.

Laboratory measurements

Venous blood samples were taken on day 1 and day 14 to assess lipid profile, liver function and high sensitive C-reactive protein (hsCRP). On day 15 blood samples were taken at baseline and then for 8 h post-vaccination. Plasma and serum were then stored at -80°C . Analysis of total white cell (WCC), neutrophil count and hsCRP was undertaken by the Addenbrooke's Hospital Biochemistry and Haematology departments using standard departmental procedures. High sensitivity IL-6 was measured in serum using high-sensitivity commercial ELISA kits (R & D Systems, Abingdon, Oxfordshire, UK). The inter-assay and intra-assay coefficients of variation were less than 7%. Apolipoprotein A-I (Apo A-I) was quantified using commercial reagent by automated immunoturbidimetry. The inter-assay and intra-assay coefficients of variation were less than 4.5%.

Statistical analysis

Data were analysed with SPSS software (Version 12.0.1; SPSS Inc, USA). Results are expressed as mean $\pm$ SE, unless otherwise stated. Logarithmic transformation was performed for skewed variables (hsCRP and IL-6) and used for further analysis. For baseline demographics and lipid profiles, unpaired, two-tailed Student's *t*-tests were used to compare differences between the two groups. The effect of vaccination (day 1 vs. day 14 vs. day 15) was assessed by two-way repeated-measures ANOVA and a custom hypothesis testing (simple) of within-subject contrasts was performed for the simvastatin vs. placebo comparison. In *post hoc* tests, the effect of individual treatments was determined using paired Student's *t*-tests with Bonferroni's adjustment for two comparisons. Pearson's correlations were calculated between absolute changes in lipid parameters and inflammatory markers and haemodynamic measures. A probability of <0.05 was considered significant.

Table 1

Baseline demographics and biochemical characteristics

	Placebo (<i>n</i> = 25)	Simvastatin (<i>n</i> = 25)
Gender (male/female)	14/11	10/15
Age (years)	27 $\pm$ 1	25 $\pm$ 1
Peripheral SBP (mmHg)	117 $\pm$ 2	117 $\pm$ 2
Peripheral DBP (mmHg)	72 $\pm$ 2	73 $\pm$ 1
Heart rate (beats min ⁻¹)	67 $\pm$ 3	66 $\pm$ 2
Height (m)	1.74 $\pm$ 0.2	1.72 $\pm$ 0.2
Weight (kg)	73 $\pm$ 4	66 $\pm$ 2
Total cholesterol (mmol L ⁻¹)	4.5 $\pm$ 0.2	4.4 $\pm$ 0.1
Triglycerides (mmol L ⁻¹)	1.2 $\pm$ 0.1	1.0 $\pm$ 0.1
HDL (mmol L ⁻¹)	1.59 $\pm$ 0.10	1.76 $\pm$ 0.09
LDL (mmol L ⁻¹)	2.49 $\pm$ 0.14	2.34 $\pm$ 0.10
TC : HDL ratio	2.91 $\pm$ 0.18	2.57 $\pm$ 0.12
Apo A-I (mg dl ⁻¹)	144.9 $\pm$ 6.3	133.6 $\pm$ 9.1
White cell count (10 ⁹ l ⁻¹)	6.5 $\pm$ 0.3	6.5 $\pm$ 0.4
Neutrophil count (10 ⁹ l ⁻¹)	3.9 $\pm$ 0.3	4.3 $\pm$ 0.4
hsCRP (mg l ⁻¹)	2.3 $\pm$ 1.3	1.3 $\pm$ 1.2
IL-6 (pg ml ⁻¹)	1.29 $\pm$ 0.18	1.22 $\pm$ 0.17

Values are represented as mean $\pm$ SE. Apo A-I, apolipoprotein A-I; DBP, diastolic blood pressure; hsCRP, high sensitive C-reactive protein; HDL, high density lipoprotein; IL-6, interleukin-6; LDL, low density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TC : HDL ratio, total cholesterol : high density lipoprotein ratio.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Results

The baseline characteristics of the two groups are shown in Table 1. There was no difference in baseline demographics, lipid profile, WCC, neutrophil count, hsCRP, IL-6 or Apo A-I between the simvastatin and placebo groups. There were no reported adverse events.

Lipid analyses

Following the treatment phase (day 1 vs. day 14), there was a significant reduction in total cholesterol (mean change of -0.9 ± 0.1 mmol L⁻¹, $P < 0.0001$), low density lipoprotein

Table 2

Change in full lipid profile and CRP following 14 day treatment phase

	Placebo	Simvastatin	Significance between groups
Total cholesterol (mmol L ⁻¹)	-0.1 ± 0.1	-0.9 ± 0.1*	0.001
Triglycerides (mmol L ⁻¹)	-0.1 ± 0.1	-0.3 ± 0.2†	0.01
HDL (mmol L ⁻¹)	-0.06 ± 0.01	-0.05 ± 0.01	>0.05
LDL (mmol L ⁻¹)	-0.05 ± 0.1	-0.73 ± 0.10*	<0.0001
TC/HDL ratio	-0.1 ± 0.1	-0.4 ± 0.1*	0.003
CRP (mg L ⁻¹)	0.17 ± 0.22	0.01 ± 0.33	0.7

Values are expressed as mean change ± SE (day 1 vs. day 14). Significance was measured using one-way ANOVA to assess group comparisons. For changes in response to treatment within groups, paired two-tailed Student's *t*-tests were used. Significance is indicated by * ($P < 0.0001$), † ($P < 0.05$). CRP, C-reactive protein; HDL, high density lipoprotein; LDL, low density lipoprotein; TC, total cholesterol; TC : HDL ratio, total cholesterol : high density lipoprotein ratio.

Table 3

Change in inflammatory response following vaccination

	Placebo	Simvastatin	Between groups
Body temperature (°C)	0.1 ± 0.2	0.1 ± 0.1	0.9
White cell count (10 ⁹ L ⁻¹)	2.9 ± 0.4†	3.1 ± 0.3†	0.7
Neutrophil count (10 ⁹ L ⁻¹)	2.7 ± 0.4†	3.5 ± 0.5†	0.2
hsCRP (mg L ⁻¹)*	0.2 ± 0.1	0.1 ± 0.2	0.8
IL-6 (pg mL ⁻¹)*	6.7 ± 0.8†	7.1 ± 0.9†	0.7
Apo A-I (mg dL ⁻¹)	-6.0 ± 2.6‡	3.7 ± 3.9	0.04

Values are expressed as mean change ± SE [baseline (0 h) vs. 8 h post-vaccine]. For changes in inflammatory response to vaccination within subjects, paired two-tailed Student's *t*-tests were used and for group comparisons unpaired Student's *t*-tests were performed. *Logarithmic transformation was performed for skewed variables and used for further analysis. Significance is indicated by † ($P < 0.001$), ‡ ($P < 0.05$). Apo A-I, apolipoprotein A-I; hsCRP, high sensitive C-reactive protein; IL-6, interleukin-6.

(-0.73 ± 0.10 mmol L⁻¹, $P < 0.0001$) total cholesterol : high density lipoprotein ratio; (-0.43 ± 0.1 , $P < 0.001$) and triglycerides (-0.3 ± 0.2 mmol L⁻¹, $P = 0.04$) in the simvastatin group, but HDL cholesterol did not change significantly following simvastatin. There was no change in lipid profile in the placebo group (Table 2). The changes in TC, LDL and TC : HDL ratio were significantly different between the simvastatin and placebo group after the treatment phase (Table 2)

At 8 h after vaccination (day 15: baseline vs. 8 h) there was a significant reduction in Apo A-I in the placebo group (144.9 ± 6.3 mg dL⁻¹ vs. 138.9 ± 5.5 mg dL⁻¹, 95% CI of the difference -11.5 , -0.5 mg dL⁻¹, $P = 0.03$). However, there was no change in the simvastatin group (133.6 ± 9.1 mg dL⁻¹ vs. 137.3 ± 9.5 mg dL⁻¹, 95% CI of the difference -4.5 , 11.9 mg dL⁻¹, $P > 0.05$). There was a significant difference in Apo A-I changes between the two groups (-6.0 ± 2.6 vs. 3.7 ± 3.9 , $P = 0.04$) (Table 3).

Arterial stiffness and endothelial function response to pre-treatment with simvastatin

Following the 14 day treatment phase (day 1 vs. day 14) there was no change in aPWV in the placebo (5.84 ± 0.88

vs. 5.80 ± 0.87 m s⁻¹, $P = 0.7$) or in the simvastatin group (5.67 ± 0.72 vs. 5.68 ± 0.73 m s⁻¹, $P = 0.9$). Neither was there a change in FMD response following placebo (6.66 ± 3.80 vs. $6.77 \pm 4.10\%$, $P = 0.7$) or simvastatin (6.33 ± 3.43 vs. $7.07 \pm 3.94\%$, $P = 0.2$) (Table 4).

Inflammatory and cytokine response to vaccination

Following the vaccination (day 15: baseline vs. 8 h post-vaccination), there was a significant increase in total WCC, neutrophil count and IL-6 in both groups (Table 3). CRP concentrations and body temperature did not change significantly in the 8 h.

Endothelial function response to vaccination

Baseline brachial artery diameter did not differ between the two groups (3.63 ± 0.14 vs. 3.87 ± 0.16 mm, $P > 0.05$) and there was no difference in baseline brachial artery diameter between FMD and GTN measurements throughout the study, demonstrating a return to baseline diameter following reactive hyperaemia. There was no change in brachial artery diameter before and after the treatment phase with simvastatin or placebo (day 1 vs. day 14). Similarly, there was no change in baseline diameter following vaccination (day 14 vs. day 15) in either the placebo or simvastatin group (3.65 ± 0.15 vs. 3.62 ± 0.15 mm, $P > 0.05$) and 3.81 ± 0.17 vs. 3.78 ± 0.16 mm, $P > 0.05$, respectively). Following vaccination (day 14 vs. day 15) there was a significant reduction in FMD response compared with baseline in the placebo group (6.77 ± 4.10 vs. $5.27 \pm 2.89\%$, 95% CI of the difference -2.49 , -0.52 , $P = 0.03$). However, this effect was not observed in the simvastatin group (7.07 ± 4.37 vs. $7.17 \pm 3.94\%$, 95% CI of the difference -1.1 , 1.3 , $P > 0.05$) (Figure 2). There was a significant difference between the change in FMD response in the placebo vs. simvastatin group ($P < 0.001$). There was no change in the response to sublingual GTN throughout the study in either group (Figure 2).

Table 4

Haemodynamic parameters at baseline, following simvastatin/placebo and typhoid vaccination

		Baseline Day 1	Day 14	8 h post-vaccine Day 15	Significance Overall	Between groups
MAP (mmHg)	Placebo	83 ± 9	85 ± 7	82 ± 7	0.3	0.5
	Simvastatin	83 ± 7	83 ± 7	81 ± 6	0.3	
AP (mmHg)	Placebo	2 ± 8	2 ± 5	2 ± 4	0.4	0.4
	Simvastatin	3 ± 7	1 ± 4	1 ± 5	0.04	
Alx (%)	Placebo	7 ± 14	7 ± 13	5 ± 12	0.5	0.8
	Simvastatin	8 ± 11	5 ± 13	4 ± 16	0.2	
HR (beats min ⁻¹)	Placebo	65 ± 10	66 ± 11	62 ± 9	0.1	0.03
	Simvastatin	60 ± 9	65 ± 11	64 ± 10	0.03	
bPWV (m s ⁻¹)	Placebo	7.91 ± 1.07	7.82 ± 0.94	8.0 ± 1.02	0.4	0.4
	Simvastatin	7.73 ± 0.94	7.90 ± 0.92	7.8 ± 1.02	0.6	
aPWV (m s ⁻¹)	Placebo	5.84 ± 0.88	5.80 ± 0.87	6.21 ± 0.97†	0.002	0.016
	Simvastatin	5.67 ± 0.72	5.68 ± 0.73	5.72 ± 0.74	0.9	
FMD (%)	Placebo	6.66 ± 3.80	6.77 ± 4.10	5.27 ± 2.88*	0.03	<0.001
	Simvastatin	6.33 ± 3.43	7.07 ± 4.37	7.17 ± 3.94	0.9	

Values represent means ± SD. Significance measured using one-way repeated measures ANOVA. When overall test was significant ($P < 0.05$), within-subject contrasts were made and significance is indicated * $P < 0.05$, † $P < 0.01$ (day 14 vs. day 15). Paired t -test was used to test the effect of individual treatments. MAP, mean arterial pressure; AP, augmentation pressure; Alx, augmentation index; bPWV, brachial pulse wave velocity; aPWV, aortic pulse wave velocity.

Figure 2

Effect of typhoid vaccination on aortic pulse wave velocity in the simvastatin and placebo groups. Measurements were taken on day 15, before and 8 h following a typhoid vaccination. Bars represent mean and SE, $n = 50$. Pre-vaccination (□); 8 h post-vaccination (■)

Arterial stiffness and haemodynamic responses to vaccination

There were no differences in peripheral, central or mean blood pressure, augmentation pressure, Alx, bPWV and aPWV between the two groups at baseline (Table 4) or following the 14 day treatment phase (day 1 vs. day 14). Eight hours after vaccination (day 14 vs. day 15) there was a $0.40 \pm 0.51 \text{ m s}^{-1}$ increase in aPWV in the placebo group (95% CI of the difference 0.19, 0.62, $P = 0.002$) but no

Figure 3

Effect of typhoid vaccination on flow mediated dilatation in the simvastatin and placebo groups. Measurements were taken on day 15, before and 8 h following a typhoid vaccination. Bars represent mean and SE, $n = 50$. Pre-vaccination (□); 8 h post-vaccination (■)

change in the simvastatin group ($0.04 \pm 0.54 \text{ m s}^{-1}$, 95% CI of the difference $-0.19, 0.27$, $P = 0.96$). There was a significant difference when comparing between the two groups ($P = 0.016$). There was no difference in augmentation pressure, mean arterial pressure, heart rate, Alx, or bPWV following typhoid vaccine in either group (see Table 4 and Figure 3).

When looking at the relationship between changes in aPWV and endothelial function following the vaccination, we found no correlation between these two indices either

analysing the data by treatment group; (simvastatin, $r = -0.03$, $P = 0.9$, placebo, $r = 0.43$, $P = 0.1$) or pooling the data ($r = 0.1$, $P = 0.4$). Neither were there correlations between changes in aPWV or FMD and in IL-6, Apo A-I and CRP following the vaccination.

Discussion

This study demonstrates that typhoid vaccination leads to acute aortic stiffening and endothelial dysfunction. Moreover, for the first time, we have shown that a pre-treatment with simvastatin can prevent inflammation-induced aortic stiffening, as well as endothelial dysfunction.

The use of vaccination to induce transient systemic inflammation in healthy subjects has been well described [10, 15, 16], and provides a useful experimental model of acute inflammation. In the present study vaccination with *Salmonella typhi* led to a significant 0.40 m s^{-1} increase in aPWV in the placebo group by 8 h. The magnitude of this effect is identical to that reported by Vlachopoulos *et al.* [11], and was not accompanied by any change in mean arterial pressure or heart rate, important potential confounding factors of PWV. Interestingly, we did not detect any change in brachial PWV, suggesting preferential stiffening of the larger more elastic vessels such as the aorta, rather than the peripheral muscular arteries.

In the present study there was no change in Alx, a composite measure of wave reflection and systemic arterial stiffness, following vaccination. This is in contrast to the observations of Vlachopoulos *et al.* who noted a 5% reduction in Alx, and attributed this to systemic vasodilatation [11]. Our data suggest that any such effect may have been offset by the increase in wave speed in the central arteries. The net effect would therefore be no measured change in Alx. This view is supported by our recent observations that anti-TNF- α therapy in patients with rheumatoid arthritis does not alter Alx, nor is Alx increased in these subjects, despite having a much higher aPWV than controls [20].

As expected, we also observed a concomitant, significant, 23% reduction in FMD following vaccination in the placebo group, but no change in GTN response, indicating acute endothelial dysfunction. We have previously demonstrated a correlation between endothelial function and aortic stiffness in healthy subjects [21], and that endogenous nitric oxide, in part, regulates large artery stiffness [22]. This led us to hypothesize that typhoid vaccination would lead to arterial stiffening due to reduced nitric oxide bioavailability. However, in the present study we were unable to demonstrate any correlation between vaccine-induced changes in endothelial function and aortic stiffness, suggesting that endothelial function and aortic stiffness change in parallel in response to an acute inflammatory stimulus, and are not mechanistically linked.

Several pleiotropic effects of statins have been described, including their ability to prevent endothelial

dysfunction induced by both *Salmonella typhi* vaccination and lipopolysaccharide administration [15, 16]. Our data extend these observations, to show for the first time that pre-treatment with simvastatin, not only prevents endothelial dysfunction, but also the aortic stiffening following experimental acute inflammation. Surprisingly, although a 14 day therapy with simvastatin resulted in a significant reduction in total and LDL cholesterol, this was not accompanied by any improvement in endothelial function or indices of arterial stiffness. This may seem unexpected, given that statins has been shown to improve endothelial function [23, 24] and reduce arterial stiffness [25–27] in numerous patient groups. However, our 'negative' observations may be due to the relatively small sample size.

In order to explore the potential mechanisms underlying the vascular changes we assessed the systemic inflammatory responses to the vaccination by measuring total WCC, neutrophils, IL-6, and CRP. IL-6, WCC and neutrophils counts were highest at 8 h post-vaccination. Interestingly, these effects did not differ between treatment groups, suggesting that simvastatin did not alter the inflammatory response to vaccination, but prevented the deleterious effects of inflammation on the vasculature. In contrast, CRP did not change post-vaccination and may reflect the 'downstream' nature of this acute phase reactant and the fact that the time course of our study was too short to detect changes in CRP. These data replicate the results of our previous study, in which we could not demonstrate a rise in serum CRP 8 h post-vaccination [15].

We also assessed the effects of typhoid vaccination on lipid sub-fractions including Apo A-I. Apo A-I is inversely associated with increased cardiovascular risk [28, 29] and known to have anti-inflammatory [30] and anti-oxidative properties [31]. Indeed, acute infusion of re-combinant Apo A-I improves endothelial function in patients with hypercholesterolaemia [32]. Hyka *et al.* demonstrated that Apo A-I has an anti-inflammatory function in chronic inflammatory conditions [30]. Moreover, low concentrations of Apo A-I are associated with acute inflammation, which is due to either viral or bacterial infection [33]. Our data demonstrate a significant reduction in Apo A-I after vaccination in the placebo group, but no change in the simvastatin group. Changes in Apo A-I could potentially explain the reduced endothelial function and aortic stiffening seen in the placebo group. The finding that simvastatin prevents these deleterious vascular changes after the vaccination suggests an interesting possibility that simvastatin can prevent inflammation-induced reduction in Apo A-I and thereby protect the vasculature against changes. More studies are needed to expand on our findings on Apo A-I and use of statins to reduce vascular complications associated with inflammatory conditions including rheumatoid arthritis, atherosclerosis and sepsis.

Statins, along with their primary lipid-lowering role, have been associated with numerous pleiotropic actions, such as improvement of endothelial function [23],

increased NO bioavailability [34, 35], anti-oxidant [36] and anti-inflammatory effects [37, 38]. Whether our data are representative of pleiotropic effects of statins is unclear, because we did not employ a control lipid-lowering agent. However, fibrates [39], acyl-CoA : cholesterol acyltransferase (ACAT) inhibitors [40] and LDL-apheresis [41] have all been shown to improve endothelial function, and fibrates [39] and ACAT inhibitors [40] also reduce TNF- α concentrations in hypercholesterolaemic subjects.

A major limitation of this study is that the exact time course of endothelial dysfunction and arterial stiffening following vaccination is unknown as we only measured aPWV and FMD at before and 8 h post-vaccination. Also a mock vaccination could have been given to clarify changes seen with typhoid vaccination. Moreover, although vaccination is associated with vascular changes, there is no evidence that it increases cardiovascular risk [42]. However, this is a model of acute inflammation and has been used previously to assess the impact of inflammation on arterial stiffening and endothelial function.

These experimental data suggest that aortic stiffening and endothelial dysfunction caused by acute inflammatory insult may be amenable to pharmacological intervention with statins. To this end, it is important to identify mechanisms which underpin this process and to explore further the cause and effect relationship by which acute inflammation leads to increased aortic stiffness and endothelial dysfunction. Nevertheless, we have shown an important protective effect afforded by statin therapy, and demonstrated that it protects against a decrease in Apo A-I following acute inflammation insult. Our data do not support a direct anti-inflammatory role of simvastatin, but suggest an effect on Apo A-I, a lipid fraction with anti-inflammatory and anti-oxidative functions.

Competing interests

There are no competing interests to declare.

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